Distribution and Abundance of Insecticide Resistant Greenbugs (Homoptera: Aphididae) and Validation of a Bioassay to Assess Resistance

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ABSTRACT Laboratory bioassays were conducted to determine the toxicity of four insecticides (ethyl parathion, chlorpyrifos, malathion, and carbofuran) to insecticide-susceptible and resistant populations of greenbug, *Schizaphis graminum* (Rondani). These bioassays were used to develop and validate a discriminating concentration for assessing insecticide resistance in greenbug populations in the field. Samples from wheat and sorghum in two states, Oklahoma and Kansas, indicated that insecticide resistance persists in greenbug populations over a large area at a low level.

KEY WORDS greenbug, insecticide, resistance, wheat, sorghum

THE GREENBUG, *Schizaphis graminum* (Rondani), is an important aphid pest of small grains, including wheat and grain sorghum. Control methods for greenbugs have included natural enemies, crop cultivars resistant to the pest, and chemical insecticides. The ability of greenbug populations to overcome host plant resistance has resulted in the selection of insecticide-resistant strains.

In the greenbug, organophosphate resistance results from detoxification of the insecticide by esterases (primarily by sequestering the insecticide) and reduced acetylcholinesterase sensitivity (Siegfried and Ono 1993, Ono et al. 1994). When single aphid homogenates were electrophoresed in native polyacrylamide gels and the gels were stained for nonspecific esterase activity, individuals with one of two different elevated esterase phenotypes (pattern 1-R1 and pattern 2-R2) were identified (Shufran et al. 1996). These elevated esterase polymorphisms have been characterized and associated with different levels of resistance. Susceptible (S) insects show no elevated esterase activity or resistance to insecticides (Shufran et al. 1997a). A laboratory derived cross of R2 females and R1 males resulted in a novel phenotype identified as pattern 3 (R3). The R3 clone has a combination of the R1 and R2 elevated esterase polymorphisms (Rider et al. 1998). No R3 individuals have been identified from the field.

Greenbugs with the R1 phenotype display a single slowly migrating elevated esterase that confers up to 50-fold resistance to parathion (an organophosphate insecticide), whereas individuals of the R2 phenotype they display a series of three fast-moving esterases with elevated activity and up to 200-fold resistance to parathion (Shufran et al. 1996, Rider and Wilde 1998). Individuals of the R3 phenotype display a series of four esterases with elevated activity and 134-fold tolerance to parathion (Rider and Wilde 1998).

The development of procedures for documenting pest resistance to pesticides is a prerequisite to the successful implementation of essentially all resistance management strategies (ffrench-Constant and Roush 1991). Thus, early stages in this process include developing appropriate sampling and bioassay procedures for detecting resistance, verification of the reliability of those procedures in the field, and use of these procedures to record susceptibility levels in the location of interest. These monitoring techniques then provide a measure of assessing the genetic potential for resistance to occur and allow resistance profiles to be developed. Also, at the individual grower level, ineffective and wasted applications can be avoided. These techniques also can be used to determine if resistance or some other factors, such as ineffective application, are responsible for ineffective control or so-called insecticide failures (ffrench-Constant and Roush 1991).

To achieve those objectives, various methods have been developed for specific pests, including a petri dish residual bioassay for aphids (McKenzie et al. 1993), an adult and neonate larval vial testing procedure for *Helicoverpa armigera* (Hübner) (Ahmad et al. 1997), and pheromone trap assays for several lepidopterous pests on apples (Robertson et al. 1990). At Kansas State University, we have cooperated with others in conducting greenbug insecticide resistance surveys for several years (Shufran et al. 1997b). We have used polyacrylamide gel electrophoresis (PAGE) to characterize the potential insecticide re-

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Table 1. Response of four populations of adult apterous greenbugs to four insecticides using a petri dish bioassay

Insecticide	Colony	n	LC_{50} (95% CI)[a]	Slope ± SE	χ^{2b}
Parathion	S	240	4.5 (1.2–14.6)a	0.8 ± 0.2	18.4*
	R1	3070	641.3 (479.4–817.6)b	1.2 ± 0.1	139.4*
	R2	2820	2397.0 (1834.0–3174.0)c	1.9 ± 0.3	47.2*
	R3	2620	2082.0 (1315.0–3203.0)c	2.2 ± 0.5	19.4*
Malathion	S	620	4.6 (2.5–7.4)a	1.1 ± 0.1	58.9*
	R1	650	56.2 (45.6–69.6)b	1.6 ± 0.1	218.6*
	R2	670	41.1 (29.0–58.7)b	1.9 ± 0.2	69.0*
	R3	330	40.2 (21.7–73.7)b	1.0 ± 0.1	50.5*
Lorsban	S	240	0.5 (0.2–0.8)a	1.1 ± 0.2	27.7*
	R1	260	6.7 (3.6–13.3)b	1.5 ± 0.3	32.7*
	R2	270	40.6 (21.4–82.8)c	1.0 ± 0.2	44.0*
	R3	240	15.9 (7.1–35.8)bc	1.1 ± 0.2	34.5*
Furadan	S	525	0.7 (0.4–1.1)a	1.3 ± 0.2	55.9*
	R1	530	3.0 (2.3–4.0)b	1.8 ± 0.2	110.2*
	R2	535	2.8 (2.1–3.8)b	1.6 ± 0.2	117.5*
	R3	510	2.4 (1.7–3.3)b	1.7 ± 0.2	93.4*

S, colony with no elevated esterase or susceptible; R1, esterase pattern 1 colony; R2, esterase pattern 2 colony; R3, esterase pattern 3 colony.

[a] LC_{50}s are expressed in ppm of insecticide; 95% confidence intervals were calculated using PROC PROBIT (SAS Institute 1998); Criterion for significant difference between colonies is no overlap in 95% confidence intervals; LC_{50}s followed by the same letter are not significantly different.

[b] Chi-square significant ($P < 0.05$).

sponse of individual greenbugs or populations of greenbugs. Although this technique is highly accurate, the cost, equipment, and time required preclude its use in the field and prevent a rapid turn-around time.

The objectives of this study were to establish baseline susceptibility to four insecticides commonly employed to control greenbugs, determine and validate a diagnostic concentration that could rapidly be used to assess resistance in greenbugs from field collected populations, and to use the diagnostic concentration to quantify and further document the occurrence of insecticide-resistant greenbug populations in Kansas and Oklahoma.

Materials and Methods

Colonies of four different greenbug strains were established from field collections or greenhouse colonies from sorghum in Kansas. A R1 resistant, R2 resistant, R3 resistant, and susceptible colony (Shufran et al. 1996, Rider et al. 1998) were reared on Deltapine (Eagan, MN) 550E grain sorghum in isolated cages in a greenhouse at 25 ± 3°C and a photoperiod of 16:8 (L:D) h.

Bioassays. Insecticides were applied to the top and bottom of plastic petri dishes (5.0 by 0.8 cm) (VWR Scientific Products, Batavia, IL) in 500 μl of 95% ethyl alcohol (1 ml/dish). After the alcohol had been applied to the dish, the dish was rotated by hand to ensure even distribution of the liquid and allowed to dry in a fume hood for 2 h. The top and bottom of each dish then were put together, placed in an airtight self-sealing plastic bag, and stored at −20°C until used for testing. Commercial formulations of ethyl parathion (8E), malathion (2E), carbofuran (4F), and chlorpyrifos (4E) were used. Each insecticide bioassay consisted of four concentrations that resulted in a range of >0 and <100% mortality for each colony. Four dishes (replicates) were used for each of the concentrations and 10 adult insects were placed in

Table 2. Comparison of discriminating concentration (DC) and polyacrylamide gel electrophoresis (PAGE) tests for greenbug resistance during 1996 and 1997

Test	Year	No. of samples	Mean % resistant	Range
DC	1996	25	3.4	0–30
PAGE	1996	25	2.3	0–20
DC	1997	22	11.1	0–50
PAGE	1997	22	11.5	0–70

[a] Pearson correlation coefficient (r^2) = 0.81 (SAS Institute 1988).

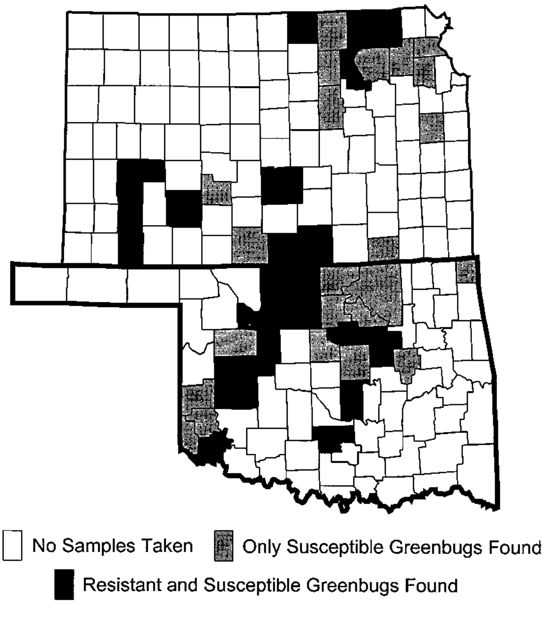

Fig. 1. Combined results of the 1996 greenbug insecticide resistance survey from wheat and sorghum.

Table 3. Results of the 1996–1999 greenbug insecticide resistance survey in wheat and sorghum

State	Year	Crop	No. of samples	Mean % resistant	Range
Oklahoma	1996	Wheat	59	2.0	0–30.0
		Sorghum	4	0.0	0
Kansas	1996	Wheat	6	3.5	0–15.0
		Sorghum	33	3.1	0–22.5
Oklahoma	1997	Wheat	26	27.2	0–63.0
		Sorghum	14	0.0	0
Kansas	1997	Wheat	0	—	—
		Sorghum	21	0.6	0–75.0
Oklahoma	1998	Wheat	11	0.0	0
		Sorghum	12	0.0	0
Kansas	1998	Wheat	0	—	—
		Sorghum	30	3.1	0–30.4
Oklahoma	1999	Wheat	2	0.0	0
		Sorghum	13	0.0	0
Kansas	1999	Wheat	0	—	—
		Sorghum	26	3.0	0–40.0

each dish with an artist's paintbrush. Mortality was assessed after 2 h by probing insects with a paintbrush. Lack of coordinated movement was the criterion for death. The entire procedure was repeated at least three times on different days.

Data were analyzed using PROC PROBIT (SAS Institute 1988) with the C option to adjust for control mortality. LC_{50} values were considered significantly different if the 95% confidence intervals did not overlap. These bioassays were used to estimate a discriminating concentration.

Field Surveys. Greenbugs were collected from the field in 1996–1999 throughout Kansas and Oklahoma by county agents, consultants, and university researchers who cut leaves from plants infested with greenbugs. Leaf sections with live greenbugs were shipped by overnight mail to Kansas State University and placed on sorghum plants immediately. In 1996 and 1997, samples from 25 and 33 locations, respectively, were split into two groups. One group was characterized by PAGE (at least 20 greenbugs per sample), and its counterpart characterized by a discriminating concentration of parathion in petri dishes (10 aphids in each of four dishes). The relationship between PAGE and the discriminating concentration test was characterized using PROC CORR (SAS INSTITUTE 1988). In 1998 and 1999, only the discriminating concentration procedure was used to characterize populations since the 1996 and 1997 tests indicated that it was a reliable indicator.

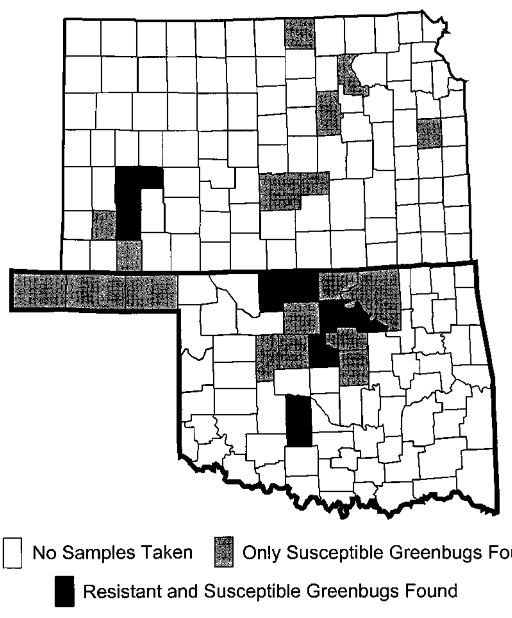

Fig. 2. Combined results of the 1997 greenbug insecticide resistance survey from wheat and sorghum.

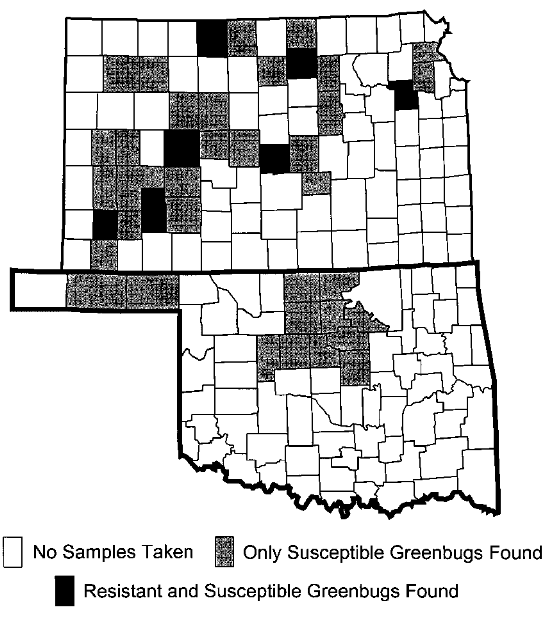

Fig. 3. Combined results of the 1998 greenbug insecticide resistance survey from wheat and sorghum.

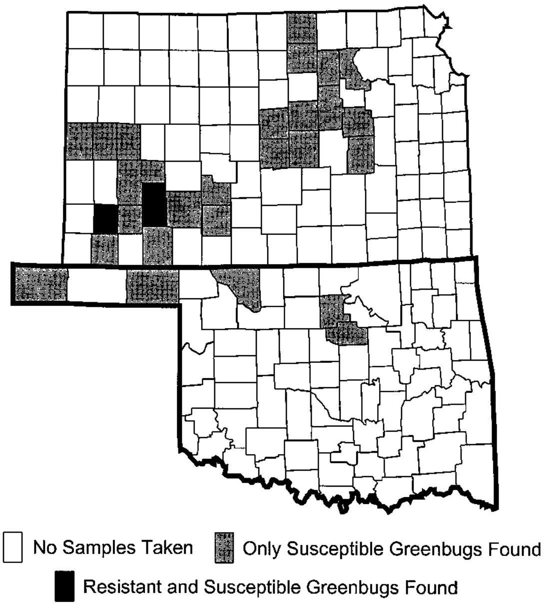

Fig. 4. Combined results of the 1999 greenbug insecticide resistance survey from wheat and sorghum.

Results

Bioassay. Responses of the susceptible and various insecticide resistant greenbug strains to parathion, chlorpyrifos, carbofuran, and malathion are shown in Table 1. The relative level of resistance (resistance ratio) of parathion was the greatest among the four insecticides tested. Therefore, a discriminating concentration of 333 ppm was chosen.

Comparison of PAGE and Discriminating Concentration. Results of the PAGE and discriminating concentration petri dish test comparison showed that there was a good correlation between these two methods in both 1996 and 1997 ($r^2 = 0.81$) (Table 2). The discriminating concentration test is not as precise as the PAGE test because it does not detect the form of resistance (R1, R2, R3), but its advantages in time and money saved make this a worthwhile and valuable procedure. The petri dish bioassay is relatively inexpensive. Although materials and labor may vary, supplies averaged $1.50 per bioassay and labor averaged 2.5 h to complete a bioassay, including petri dish preparation time. The ease of using the petri dish test lends itself for use in field monitoring of insecticide resistance because it is economical and reliable, yields quick results, and easily can be incorporated into integrated pest management programs.

Results of Field Surveys. There were 102 samples submitted (65 from wheat and 37 from sorghum) in 1996. Although greenbug insecticide resistance was found in several widespread areas (Fig. 1) the average percentage of resistant individuals was quite low (Table 3). There were 61 samples submitted in 1997 (26 from wheat and 35 from sorghum). Again, insecticide resistance was found in several widespread areas (Fig. 2) but the incidence of resistance was low again, except for the samples collected from wheat in Oklahoma (Table 3). A greenbug outbreak in Oklahoma in 1997 resulted in widespread insecticide use and may have accounted for the selection of resistant populations. Similar results were seen in a survey conducted by Shufran et al. (1997b) from 1991 to 1995. There were 53 samples submitted in 1998 (11 from wheat and 42 from sorghum). The incidence of insecticide resistance remained low (Table 3) although widely distributed (Fig. 3). There were 41 samples submitted in 1999 (two from wheat and 39 from sorghum). The presence of insecticide-resistant individuals was confined to two counties in the southwest corner of Kansas (Fig. 4) and the overall incidence of greenbug insecticide resistance remained low (Table 3).

Our overall survey results from the four years of sampling agree with those of Shufran et al. (1997b). The incidence of insecticide resistance was low in all populations except when widespread application of insecticides occurred, such as what occurred in Oklahoma wheat fields in 1997. Shufran et al. (1997b) and Stone et al. (2000) have addressed the various biological factors that may affect the distribution, abundance, and relative fitness of greenbug resistant individuals. Their studies suggest that the R1 phenotype have a lower net reproductive rate than the susceptible and R2 phenotypes. The results obtained in this study and the development of a quick and reliable discriminating concentration bioassay should allow those involved in pest management decisions with this pest on both sorghum and wheat to select insecticides based on known susceptibility of aphids present in individual fields (Archer et al. 1994). Several possible management strategies were discussed by Shufran et al. (1997a). Planting imidacloprid-treated sorghum seed may be the best method to control potentially resistant greenbugs in areas where winter wheat was treated for greenbug infestations (Sloderbeck et al. 1996).

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